DISORDER/SETTING

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DISORDER/SETTING

Question 1: What is the specific clinical disorder to be studied?

The specific clinical disorder is primary iron overload of adult onset sufficient to cause significant morbidity and mortality.

- *Iron overload* refers to excess deposition of iron in parenchymal cells in the liver, pancreas and heart, and/or increased total body mobilizable iron.
- *Primary* refers to a genetically determined abnormality of iron absorption, metabolism, or both.
- *Morbidity* refers to organ damage that results in physical disability over and above that seen in the absence of iron overload.

A single inherited disorder, *HFE*-related hereditary hemochromatosis (HHC) accounts for the vast majority of cases of primary iron overload in Caucasian adults in the United States. The *HFE* gene is linked to HLA-A on the short arm of chromosome 6. *HFE*-related HHC is a recessive disorder. A small proportion of primary iron overload cases is explained by inherited disorders other than *HFE*-related HHC. Juvenile hemochromatosis (*HFE2*) is a rare autosomal recessive disorder associated with a gene mapped to the long arm of chromosome 1. Iron overload occurs earlier, with patients having a more severe clinical presentation in the second and third decades. A third type of autosomal recessive, non-*HFE*-related primary iron overload results from mutations in the transferrin receptor 2 gene (*TfR2)* located on chromosome 7, and has been reported in a few Italian families (Camaschella *et al.*, 2000; Girelli *et al.*, 2002). A fourth reported type shows autosomal dominant transmission and is associated with mutations in the ferroportin gene (*SLC11A3*) on the long arm of chromosome 2. Other rare inherited disorders of iron metabolism include atransferrinemia, hyperferritinemia, and aceruloplasminemia (Fletcher and Halliday, 2001). Although iron absorption is enhanced in a number of other inherited disorders (e.g., thalassemia major, iron-loading anemias, hereditary spherocytosis), these are considered secondary, or acquired, forms of iron overload and are not considered in this report.

The original clinical diagnosis of hereditary hemochromatosis was based on the triad of hepatic cirrhosis, diabetes mellitus, and skin pigmentation. However, by this point in the natural history of the disease, tissue damage due to iron overload has progressed too far for treatment to be more than palliative. If population screening for iron overload were to be considered, two strategies for initial testing might be employed:

- direct DNA testing for the homozygous C282Y *HFE* genotype
- biochemical measurement(s) of serum transferrin saturation

Both strategies require that those with positive screening results undergo further testing to quantify the extent of iron overload. For C282Y homozygous individuals, serum transferrin saturation and ferritin would be measured as follow-up tests. For individuals with elevated transferrin saturation, further testing would also be needed to measure extent of iron overload (ferritin) and to differentiate *HFE*-related HHC from other causes of primary iron overload. This report focuses on the first strategy employing DNA testing as the primary screening test.

DISORDER/SETTING

Question 2: What are the clinical findings defining this disorder?

In 1880, Tosier described a group of patients with the triad of hepatic siderosis and cirrhosis, diabetes mellitus, and skin pigmentation; von Recklinghausen postulated that the origin of the iron deposited in the liver was the blood and named this disorder *"hemochromatosis"*. Subsequently, it was shown that the iron deposition in HC is due to excessive absorption of iron from the gastrointestinal tract (Powell *et al.,* 1975; Valberg *et al.,* 1980). Although iron may be deposited in many tissues, the primary site of uptake is the hepatic parenchymal cells (Powell *et al.,* 1975; Worwood, 1997). The clinical findings of iron overload are all directly or indirectly due to tissue iron deposition and damage by the iron, possibly from lipid peroxidation (Gutteridge *et al.,* 1985; Britton *et al.,* 1987; Sherwood *et al.,* 1998). The following are the most commonly involved organ systems:

Liver: Early in the course of iron overload, hepatic iron deposition may result in hepatomegaly, abdominal pain, and abnormal liver function tests. If the deposition progresses and is not treated, hepatic fibrosis, cirrhosis, liver failure, and possibly hepatic carcinoma (either hepatocellular carcinoma or cholangiocarcinoma) may occur (Powell *et al.,* 1975; Niederau *et al.,* 1985; Cuthbert, 1997; Racchi *et al.,* 1999; Blanc *et al.,* 2000; Bonkowsky and Lambrecht, 2000).

Heart: Iron deposition in cardiac muscle can produce dilated or restrictive cardiomyopathy, either of which may result in heart failure (Niederau *et al.,* 1985; Niederau *et al.,* 1996). This is the second most common cause of death, after liver disease, in untreated patients. Adult males presenting with clinical disease before age 40 have a high prevalence of cardiomyopathy and arrhythmias; 60% under age 40 and nearly all under age 30 died of congestive failure (Finch and Finch., 1955). Arrhythmias are most often atrial but may be ventricular (Milder *et al.,* 1980; Niederau *et al.,* 1985; Dabestani *et al.,* 1988). Although the etiology is uncertain in most cases, iron deposits in the myocardium appear to be involved. Other factors, such as alcohol, probably exacerbate the cardiomyopathy and arrhythmias (Schellhammer *et al.,* 1967). If the patient can be kept alive by cardiotherapy during phlebotomy therapy, the cardiac disorders are completely reversible (Short *et al.,* 1981; Dabestani *et al.,* 1988).

Endocrine glands: Damage to the beta cells of the pancreatic islets, either directly from iron or from autoimmune reactions (presumably secondary to alteration of antigens by oxidation or other means), may result in diabetes mellitus (Niederau *et al.,* 1985; Adams *et al.,* 1991; Moirand *et al.,* 1997), Insulin resistance secondary to hepatic damage may also contribute to the metabolic dysfunction (Smith, 1990; Mendler *et al.,* 1999). Deposition of iron in the anterior pituitary gland may result in sexual dysfunction, including loss of libido, impotence, testicular atrophy, and amenorrhea, secondary to reduced production of the gonadotrophic hormones (Bezwoda *et al.,* 1977; Walton *et al.,* 1982).

Joints: Arthralgia or frank degenerative or inflammatory arthropathy is the single largest contributor to patient-perceived morbidity (Adams and Speechley, 1996). Although other joints may be affected, the 2nd and 3rd metacarpal joints are most commonly involved (Axford, 1991; McCurdie and Perry, 1999). Specific radiological findings include bone erosion, hooking

osteophytes, and chondrocalcinosis (Axford, 1991; Hamilton *et al*.,1981; Huaux *et al.*,1986), similar to changes seen in severe hyperparathyroidism (Huaux *et al.*, 1986).

Skin: Bronze skin pigmentation is believed to be due primarily to excessive melanin secretion (Smith, 1990; Adams *et al.*, 1997; Pounder, 1997), although iron deposition itself may also be involved.

Other: Non-specific symptoms include lethargy, weakness, chronic fatigue, emotional distress (including frank depression), and abdominal pain. These are frequently the presenting, and occasionally the only, symptoms of iron overload (Adams and Valberg, 1996; Adams *et al.*, 1997; Moirand *et al.*, 1997).

DISORDER/SETTING

Question 3: What is the setting in which the test is to be performed?

The setting for this report is population screening of adults. Several studies have proposed biochemical and/or DNA screening for all adults. The published recommendations, however, nearly always focus on the Caucasian population because of the higher prevalence of HHC and the high proportion attributable to the *HFE* gene. The recommended minimum age for screening ranges from 20 to 40 years (Question 5). Iron overload is usually not present in males until the second or third decade of life, and clinical signs and symptoms are uncommon before the fifth decade. Women generally develop iron overload and associated clinical findings 8 to 30 years later than men (Meyer *et al.*, 1990; Adams *et al.*, 1991; Edwards and Kushner, 1993; Bulaj *et al.*, 2000). Although *HFE* mutations can be reliably detected at any time during life, the low penetrance in the early decades raises both ethical and medical questions about the appropriateness of genotyping prior to adulthood (Brittenham *et al.*, 1998; Burke *et al.*, 1998; McDonnell *et al.*, 1998; Cogswell *et al.*, 1999; Bhavnani *et al.*, 2000; Hickman *et al.*, 2000; Byrnes *et al.*, 2001; Evans *et al.*, 2001).

In order for testing to be effective from a public health perspective, a screening program would need to be widely available. One possibility would be for primary care providers to offer screening as part of routine care (McDonnell *et al.*, 1998; Niederau *et al.*, 1998). However, a substantial proportion of the adult population does not avail itself of such care. For that reason, other screening strategies need to be considered, in order to reach a broader segment of the target population. For example, testing might be offered in a variety of public settings, similar to the model used for cholesterol testing.

It has been proposed that rheumatology (Olynyk *et al.*, 1994) and diabetic (O'Brien *et al.*, 1994) clinic patients be "screened" for iron overload. However, this type of routine testing cannot be considered screening. The more appropriate terminology would be "case finding," since a pre-selected, symptomatic population is being tested. Case finding will not be discussed in this report.

DISORDER/SETTING

Question 4: What DNA tests are associated with this disorder?

In 1996, Feder *et al.* reported a 250 kb region on the short arm of chromosome 6, encoding a major histocompatibility complex (MHC) class I-like protein that was mutated in a large proportion of individuals with clinically diagnosed HHC (Feder *et al.*, 1996). This gene was initially called *HLA-H* and subsequently renamed *HFE*. Two *HFE* missense mutations, C282Y and H63D, were initially described, and at least 17 other allelic variants of the *HFE* gene have now been reported (LeGac *et al.*, 2001; Beutler *et al.*, 2002). By altering *HFE* protein structure and disrupting β_2-microglobulin binding and cell surface expression, the C282Y mutation results in significant loss of protein function (Feder *et al.*, 1996; Feder *et al.*, 1997). Homozygosity for this mutation is most strongly correlated with clinically diagnosed primary iron overload due to HHC. The effects of the other common mutations, H63D and S65C, on protein function are less severe, although both are associated with milder forms of the disorder in a small proportion of individuals who also carry the C282Y mutation (compound heterozygotes). It is not yet completely clear whether H63D and S65C allelic variants are minor mutations with low penetrance or polymorphisms in linkage disequilibrium with one or more as yet unidentified mutations (Feder *et al.*, 1996; Douabin *et al.*, 1999). Homozygosity for the C282Y mutation is the dominant genotype in HFE-related HHC and for that reason, it will serve in this report as the only DNA test evaluated.

DNA-based tests for the two common mutations (C282Y, H63D) have been developed using a wide range of technologies that include the standard polymerase chain reaction (PCR)/restriction enzyme method (Feder *et al.*, 1996), multiplex ARMS PCR (Baty *et al.*, 1998; Bradley *et al.*, 1998), LightCycler PCR (Bollhalder *et al.*, 1999), multiplex PCR and capillary electrophoresis (Lubin *et al.*, 1999), heteroduplex analysis (Jackson *et al.*, 1997), high performance liquid chromatography (HPLC) (Liang *et al.*, 2001), and real-time PCR fluorescent resonance energy transfer (FRET) hybridization (Parks *et al.*, 2001). Though a wide variety of testing methodologies has been described, most laboratories reporting to the American College of Medical Genetics/College of American Pathologists Molecular Genetics Laboratory external proficiency testing program are currently using the PCR/restriction enzyme and ARMS PCR methods. In the United States, no kits have been approved by the Food and Drug Administration (FDA) for *HFE* testing, but some of these have been approved by the FDA as Analyte Specific Reagents (ASRs). Laboratories offering *HFE* mutation analysis will come under 'home brew' regulations.

Testing has been successfully performed using anticoagulated blood, buccal samples, and dried blood spots. Blood samples (obtained by venipuncture) serve as a highly reliable source of DNA and can be readily obtained in many health care settings. The method of collecting buccal cells by brush, swab or mouthwash is inexpensive and is well suited to collecting samples in primary care offices, at home, and in other non-health care settings. Blood and buccal samples are stable when transported at ambient temperature, and testing has been successfully performed on buccal lysates stored frozen for 3-4 years (Haddow *et al.*, 1999). Buccal sample failure rates are generally 1% or less, and results can nearly always be obtained from blood samples.

DISORDER/SETTING

Question 5: Are "pre-screening" tests employed (Asking a question about race/ethnicity)?

An inquiry about racial/ethnic heritage may be appropriate prior to offering *HFE* mutation analysis as a screening test. Both the population prevalence of hemochromatosis and the frequencies of the common alleles vary, depending upon race and ethnicity. On average, heterozygosity for C282Y is found in about 9 percent of Caucasians in Europe and North America, but it is almost never observed in populations from Africa, the Middle East, Asia, the Indian subcontinent, and Australasia (Merryweather-Clarke, 1997; Merryweather-Clarke, 1999; Hanson *et al.*, 2000).

DISORDER/SETTING

Question 6: Is it a stand-alone screening test or is it one of a series of screening tests?

The DNA-based testing that is used for screening individuals for predisposition to primary iron overload due to *HFE*-related HHC is a "stand-alone" test. It may be preceded in some programs by a screening question about race/ethnicity, intended to determine what individuals should be offered screening, or to provide specific information about the efficacy of testing. *HFE* mutation analysis identifies individuals who are at risk for iron overload because they are homozygous for the C282Y mutation. Follow-up testing to determine the extent of iron overload could identify individuals who may benefit treatment.

DISORDER/SETTING

Question 7: If it is part of a series of screening tests, are all tests performed in all instances (parallel) or are some tests only performed on the basis of other results (series)?

HFE testing may be preceded in some programs by a screening question about race/ethnicity, intended to determine what individuals should be offered screening or to provide specific information about the efficacy of testing.

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